

Calcium-sensing receptor- a novel target in rare forms of epilepsy

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Abstract

Recently, a rare, dominant, familial form of idiopathic epilepsy was found to result from mutation of the extracellular calcium-sensing receptor (CaSR). Interestingly, 5% of patients with juvenile myoclonic epilepsy (JME) also possess novel, dominant point mutations of CaSR. Because mutant CaSR may cause abnormal serum Ca, this was proposed to be the source of CaSR mutant related symptoms. However, based on our recent research, we believe that the function of CaSR in the brain is crucial to maintaining normal neuronal excitability, and that CaSR is a potential therapeutic target in these rare forms of epilepsy. We have shown that CaSR is present in 80% of all neocortical nerve terminals and that it signals to a voltage-sensitive, non-selective cation channel (NSCC). CaSR activation blocks NSCC and reduces excitatory transmission. Based on our data we proposed CaSR is the sensing arm of a homeostatic mechanism, promoting synaptic transmission during physiological decreases in brain extracellular [Ca] ($[Ca]_o$). These observations provide the rationale for further study of CaSR mutations as a potential rare cause of epilepsy. By determining whether the mutations increase or decrease CaSR signaling we will be in a position to propose whether CaSR antagonists or agonists are rational candidate novel antiepileptic drugs (AEDs). The project focuses on two specific aims designed to address the mechanism by which these CaSR mutations affect CaSR signaling.

1. Determine the effect of mutant CaSR on efficiency of receptor trafficking to the plasma membrane. We will track wild-type and mutant CaSR with distinct fluorescent tags, the green EGFP on the mutant receptor, and myc-tag on the wild type receptor. We will use quantitative imaging in human embryonic kidney (HEK) cells transfected with these mutant and wild type receptors to determine how much the wild-type CaSR impacts EGFP-tagged mutant CaSR trafficking. We used this approach to examine familial hypocalciuric hypercalcemia (FHH), another heterozygous disease arising from CaSR mutation. We found that mutant CaSR did not traffick to the membrane if expressed alone. However we tested if coexpression of CaSR, which dimerizes, impacted mutant trafficking. With this simple but innovative approach we found that heterodimer formation rescued mutant membrane expression. We will use the same approach to determine if the idiopathic epilepsy-associated CaSR mutants are expressed on plasma membrane and whether these mutants are modulated by wild-type CaSR trafficking in co-transfected HEK cells.

2. Determine the calcium affinity of idiopathic epilepsy-associated mutants. CaSR stimulation increases phospholipase C activity and thus intracellular [Ca] in transfected HEK cells. Thus we will test CaSR function using transfected cells identified by presence of EGFP after loading with a red-shifted Ca fluorophore. Using this approach we used a novel subtraction method to determine that the heterodimers, which make up 50% of all FHH patient CaSR, have a three-fold decreased affinity for Ca compared to wild-type homodimers. We will test the function of the idiopathic epilepsy-associated CaSR mutants expressed alone and with wild-type CaSR using the same method.

Thirty percent of patients with epilepsy have uncontrolled seizures despite treatment with currently available antiepileptic drugs. In addition to adding to our understanding of rare CaSR mutation-related forms of epilepsy, these studies in this project may provide insight into a new treatment strategy for more common seizure disorders. The data provided by funding of this pilot project will be essential to support an R01 application in 2010 to continue the next phase of the project.

We propose that epilepsy-related mutations alter membrane trafficking and Ca affinity of CaSR.